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Award Number: W81XWH-14-1-0141

TITLE:

Testing the Role of p21-Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease

PRINCIPAL INVESTIGATOR: Jonathan Chernoff, M.D., Ph.D.

CONTRACTING ORGANIZATION: Institute for Cancer Research
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                 Fort Detrick, Maryland 21702-5012

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Testing the Role of p21-Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease

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W81XWH-14-1-0141

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6. AUTHOR(S)

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E-Mail: Jonathan.Chernoff, M.D., Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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333 Cottman Avenue
Philadelphia, Pennsylvania 19111
E-Mail: osr@fccc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The major goal of this research project was to genetically and pharmacologically test the requirement of Group A PAK signaling in Nf2 deficient schwannoma genesis. We planned to accomplish this goal by (1) using our genetically intercrossed Pak1, Pak2 and a dominant negative Group A PAK transgene with our *PostnCre;Nf2$^{flox/flox}$* mouse schwannoma model in order to generate functional (ABR testing) and histological readouts. In turn these readouts will allow us to utilize a genetic approach to determine if indeed PAK signaling is essential to the development of *Nf2*-deficient schwannomas; (2) using histological and ABR readouts, we will treat *PostnCre;Nf2$^{flox/flox}$* mice with three different pharmacologic PAK inhibitors to determine if targeted PAK inhibition in a preclinical model of schwannoma genesis rescues tumor development.

We are on track to accomplish the goals we set. In the first year of the funded work, we completed crosses of *Pak1$^{-/-}$* and *Pak2$^{flox/flox}$* mice with *PostnCre;Nf2$^{flox/flox}$* mice and analyzed the effects of Pak loss on hearing and tumor growth. As we had speculated, loss of Pak1 function partly prevented hearing loss and tumor growth. Studies on Pak2 are ongoing. We also began Aim 2, treating *PostnCre;Nf2$^{flox/flox}$* mice with Pak small molecule inhibitors.

15. SUBJECT TERMS

NF2, Pak1, Pak2, PID, ABR, Pak inhibitors, mouse models, signal transduction, preclinical studies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

Neurofibromatosis type 2 (NF2) is an autosomal dominant genetic disease characterized by benign schwannomas that grow on the cranial and spinal nerves. While technically benign, the tumors are nonetheless progressive and relentless, usually resulting in death before age fifty from inoperable intracranial masses. To date, surgery remains the only effective therapy for these lesions, though this therapy is frequently associated with major morbidities, including loss of hearing. In this award we proposed to evaluate Group A Paks (Pak1 and Pak2) as a therapeutic target in NF2 by intercrossing Group A PAK deficient mice with our NF2 mouse model (*PostnCre;Nf2$^{flox/flox}$*). Briefly, we hypothesized that the knockdown of Group A Paks in Nf2 deficient mice will rescue or reduce tumorigenesis. In **Specific Aim 1**, we proposed to characterize Pak's signaling influence on NF2 *in vivo* by assessing hearing thresholds, survival rate, tumor formation and kinome analysis. In **Specific Aim 2**, we proposed to test Pak inhibitors in our NF2 tumor model in order to evaluate whether the inhibition of Pak rescues merlin function to wild-type levels by analyzing readouts generated from tumor growth and auditory brainstem response (ABR).

KEYWORDS:

NF2, Pak1, Pak2, PID, ABR, Pak inhibitors, mouse models, signal transduction, preclinical studies.

ACCOMPLISHMENTS:

(A) What were the major goals of the project?
The major goal of this research project was to genetically and pharmacologically test the requirement of Group A PAK signaling in Nf2 deficient schwannoma genesis. We planned to accomplish this goal by (1) using our genetically intercrossed Pak1, Pak2 and a dominant negative Group A PAK transgene with our *PostnCre;Nf2$^{flox/flox}$* mouse schwannoma model in order to generate functional (ABR testing) and histological readouts. In turn these readouts will allow us to utilize a genetic approach to determine if indeed PAK signaling is essential to the development of *Nf2*-deficient schwannomas; (2) using histological and ABR readouts, we will treat *PostnCre;Nf2$^{flox/flox}$* mice with three different pharmacologic PAK inhibitors to determine if targeted PAK inhibition in a preclinical model of schwannoma genesis rescues tumor development.
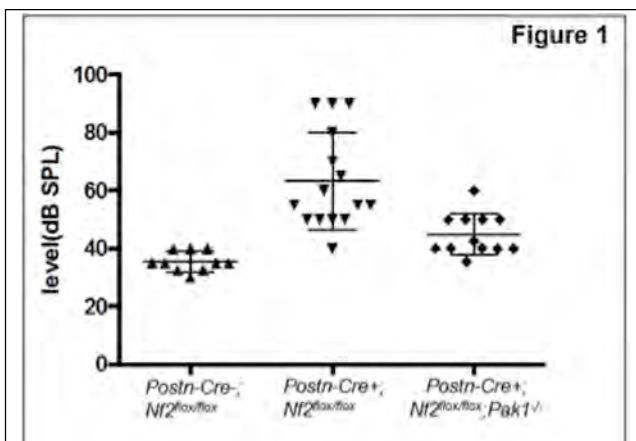

Figure 1: Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ improves hearing loss. ABR study quantitating from 9-month-old Cre-negative control Postn-Cre+;Nf2$^{flox/flox}$, Postn-Cre, and Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ mice, respectively. Each dot represents the average in left and right threshold of an individual. (P < 0.0001).

(B) What was accomplished under these goals?
1) *Major Activities:* *Postn-Cre*; *Nf2$^{flox/flox}$* mice were crossed with WT, *Pak1$^{-/-}$* and *Pak2$^{flox/flox}$* mice and the mice analyzed for hearing loss and for tumor growth.

2) *Specific Objectives:* To test the effects of *Pak1* and *Pak2* deficiency on two NF2-related pathologies: hearing loss and the growth of schwannomas.

3) *Significant results/key outcome:*
In **Specific Aim 1a.1**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) by intercrossing these mice with our NF2 mouse model, *Postn-Cre; Nf2$^{flox/flox}$* to generate the following cohorts of mice; 15 *Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{+/+}$* mice (Control), 15 *Postn-Cre+; Nf2$^{flox/flox}$; Pak1$^{+/+}$* mice (NF2-KO), 15 *Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{-/-}$* mice (PAK1-KO), and 15 *Postn-Cre+; Nf2$^{flox/flox}$; Pak1$^{-/-}$* (NF2/PAK1-DKO). These cohorts

of mice were utilized in order to assess hearing loss via auditory brainstem response (ABR) testing. Preliminary data included in the initial grant proposal indicated that our *Postn-Cre+; Nf2$^{flox/flox}$* mice showed by eight months of age, complete hearing loss, as compared to control mice, *Postn-Cre-; Nf2$^{flox/flox}$*. Functionally, the mean hearing threshold of nearly 60 dB in *Postn-Cre+; Nf2$^{flox/flox}$* mice at the age of 10 months is equivalent, as a human analog, of the inability to hear in a normal conversation, thus indicating severe disruption of an affected individual's life. In the analysis of 9 to 10 month old *Postn-Cre+; Nf2$^{flox/flox}$;Pak1$^{-/-}$* mice we observed that these mice showed improved hearing as compared 9 to 10 month old Nf2 deficient mice (*Postn-Cre+; Nf2$^{flox/flox}$* mice), as shown in **Figure 1**. Control mice (*Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{+/+}$*) showed no hearing loss at 9 to 10 months of age. In the next year, we will repeat ABR testing on 12 and 14 month old Pak1 deficient cohorts listed above and in parallel begin ABR testing on Pak2 deficient cohorts of mice intercrossed with NF2 mouse model, *Postn-Cre; Nf2$^{flox/flox}$* to generate the following cohorts of mice; 15 *Postn-Cre-; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* mice (Control), 15 *Postn-Cre+; Nf2$^{flox/flox}$* mice (NF2-KO), 15 *Postn-Cre-; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* mice (PAK2-KO), and 15 *Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* (NF2/PAK2-DKO).

In **Specific Aim 1a.2**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) in the NF2 mouse model by assessing tumor formation. Our preliminary data indicate that genetic disruption of Pak 1 or Pak 2 is not completely sufficient to inhibit tumor initiation (**Figures 2 and 3**). However, there is a strong but not statistically significant suggestion that genetic disruption of *Pak1* is resulting in a reduction in tumor size as illustrated in **Figure 2**. However, more studies will be required to verify this observation. We will continue to analyze DRG volume post-ABR testing in 12 and 14 month old *Postn-Cre+; Nf2$^{flox/flox}$;Pak1$^{-/-}$*, *Postn-Cre-; Nf2$^{flox/flox}$;Pak1-/-, Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$*, *Postn-Cre-; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* and *Postn-Cre+; Nf2$^{flox/flox}$* in the next funding period.

In **Specific Aim 1a.3**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) in our NF2 mouse model, *Postn-Cre; Nf2$^{flox/flox}$*, by assessing survival rate. Preliminary data included in the initial grant proposal indicated that our *Postn-Cre+; Nf2$^{flox/flox}$* mice
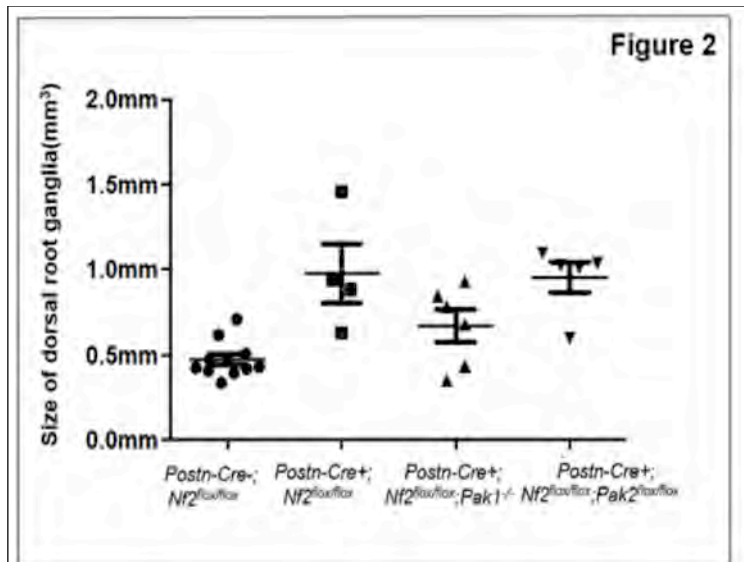

Figure 2

Figure 2: Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ decreases DRG volume. Mean size of dorsal root ganglia (DRG from 10-month-old Cre-negative control Postn-Cre+;Nf2$^{flox/flox}$, Postn-Cre, and Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ mice, respectively. Each dot represents the size of an individual DRG. The line indicates the mean size of all DRG. (P < 0.1212).
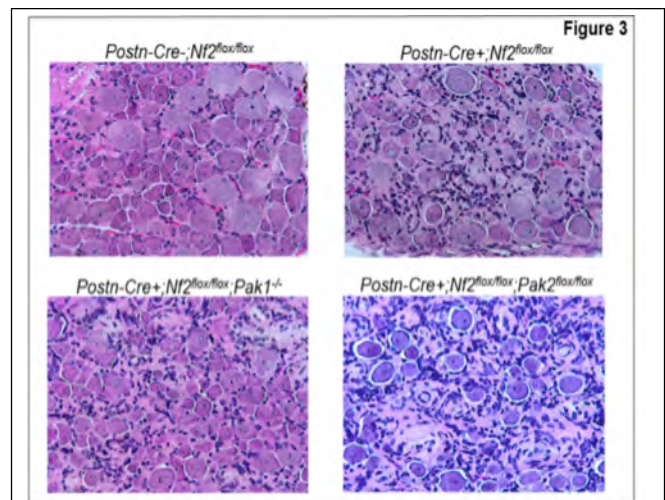
Figure 3

Figure 3: Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ slows schwannoma development. H&E stain of a (DRG from 10-month-old Postn-Cre+;Nf2$^{flox/flox}$ and Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ mice, respectively. Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ mice slowly develop schwannomas of the DRG and spinal nerves compared to Postn-Cre+;Nf2$^{flox/flox}$ mice. Diffuse spinal nerve hyperplasia and pseudo onion bulb formation of proliferating schwann cells observed in the nerve of a Postn-Cre+;Nf2$^{flox/flox}$;Pak1$^{-/-}$ mouse; however, frequency is lower. Original magnification 40x.

Figure 4: **Downregulation of Pak iosforms increases survival rate.** A kaplan-meier plot of percent survival (Y axis) as a function of time (X axis) is shown. Groups are Cre-negative control (n=15), Postn-Cre+; Nf2$^{flox/flox}$ (n=15), Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$ (n=15) and Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/- (n=15) mice, respectively.
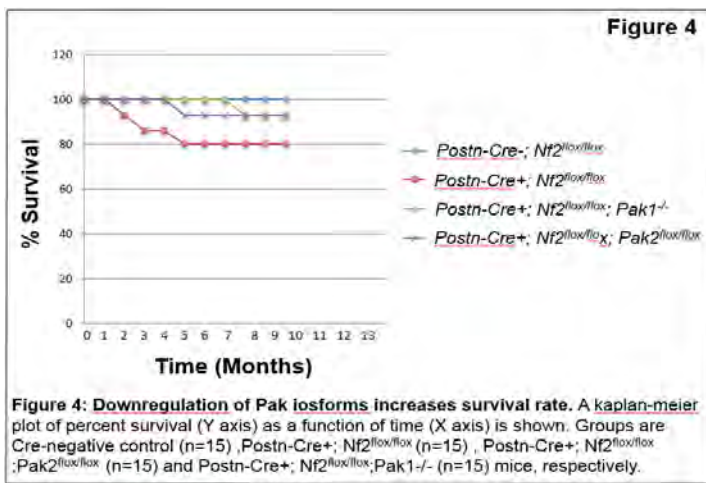
showed by eight months of age, a decrease in survival as compared to control mice, *Postn-Cre-; Nf2$^{flox/flox}$*. In the analysis 15 mice per cohort (cohorts listed in Aim1a.1). Though the study is ongoing, the preliminary data may suggest a modest improvement in survival when Pak1 or Pak2 is genetically disrupted (**Figure 4**). However, these studies are ongoing and we are currently still following these cohorts of mice. Therefore in year 2 funding we will continue to observe the, *Postn-Cre; Nf2$^{flox/flox}$ Pak1$^{-/-}$*, and *Postn-Cre; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* mouse models.

In Specific Aim 1b.1, we planned to test the effects of inhibiting all group A Pak function using a transgenic mouse that conditionally expresses the Pak Inhibitor Domain (PID), a peptide derived from Pak1 that, when expressed *in trans*, inhibits all three endogenous group A Pak isoforms. Crosses between these transgenic mice and *Postn-Cre-; Nf2$^{flox/flox}$* mice are commencing.

In **Specific Aim 1C** we proposed to assess kinome activity in tumors from the following cohorts of mice: Postn-Cre; Nf2$^{flox/flox}$ Pak1$^{-/-}$, and Postn-Cre; Nf2$^{flox/flox}$ *Pak2$^{flox/flox}$*, and ROSA26-LSL-PID mouse models. The size of each cohort will be a minimum of four mice per cohort. The 20-40 mg lysates from snap froze schwannomas will be derived from the cohorts of mice after ABR is completed. ABR testing will be completed after 14 months of age for each cohort as proposed in Aim 1. As stated in **Aim1a.2** we will collect tumor samples post ABR testing (performed in Aim1a.1) starting at 10months of age, and at 12months and at14 months. Since we have completed 10 month testing on Pak1 deficient tumor model we are currently in the process of preparing tumor lysate samples for kinome analysis in order to send to Dr. Gary Johnson's laboratory at University of North Carolina (UNC). We estimate to have these samples to Dr. Johnson in the next couple of weeks. As we continue to isolate tumors form each cohort listed in the grant application post ABR testing we will continue to prepare tumor lysate samples and send to UNC for kinome analysis during the next funding period. At this time, we have no data to report for this sub-Aim.

In **Specific Aim 2** we proposed to test small molecule Pak inhibitors on our *Postn-Cre; Nf2$^{flox/flox}$* mouse model. During the first period of funding we have begun to generate the 12 to 14 mice per group, as stated in our grant application, of our Nf2 mouse model. We will have these cohorts of mice generated and we will perform ABR testing on these mice prior to enrollment in the *in vivo* study during the next funding period. We estimate testing of these small molecule Pak inhibitors to begin before the last year of funding starts. These tumors are slow growing and it is difficult to generate the number of mice needed per group in a short period of time.

(C) What opportunities for training and professional development has the project provided?
Nothing to Report

(D) How were the results disseminated to communities of interest?
Nothing to Report

(E) What do you plan to do during the next reporting period to accomplish the goals?
In order to accomplish the goals and objects stated above, in the next funding cycle we will complete ABR testing on Pak1 cohorts, begin testing on Pak2 cohorts, continue analyzing tumor formation on Pak1 and Pak2 deficient tumor models, send out tumor samples to UNC in order to begin generating kinome readouts and begin testing small molecule Pak inhibitors on our NF2 schwannoma mouse model.

IMPACT:

Based on our recent publication in $NF2^{-/-}$ meningioma, the Pak inhibitor FRAX-1036 will likely become the reagent of choice for others analyzing group A Pak signaling in other settings (cancer, inflammation, *etc*).

CHANGES/PROBLEMS:

Nothing to Report

PRODUCTS:

Journal Publication
Chow, H.Y., Dong, B., Duron, S.G., Campbell, D., Ong, C.C., Hoeflich, K.P., Chang, L.S., Welling, D.B., Yang, Z.j., and Chernoff, J. Group I Paks as therapeutic targets in *NF2*-deficient meningioma. *Oncotarget* **10**:1981-1994, 2015. PMCID: PMC4385830
        Published; Acknowledgement of federal support: yes

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?
Please see the attached table.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Please see Other Support attached. Changes from the last reporting period are marked with a line in the right hand margin.

What other organizations were involved as partners?

> **Organization Name:** Indiana University
> **Location of Organization:** Indianapolis, IN
> **Partner's contribution to the projec***t*: Subaward
>   Indiana University has a subaward under this grant. Resources include: Facilities; Collaboration; Personnel exchanges

SPECIAL REPORT REQUIREMENTS:

Not Applicable

APPENDICES:

Not Applicable

Fox Chase Cancer Center

Name:	*J. Chernoff, MD, PhD*
Project Role:	Principal Investigator
eBRAP ID:	*chernoff*
Nearest person month worked:	*1*
Contribution to Project:	*Overall administration and guidance of research; Management and training of personnel*
Funding Support:	*N/A*
Name:	*H.-Y. Chow, PhD*
Project Role:	Research Scientist
eBRAP ID:	
Nearest person month worked:	*6*
Contribution to Project:	*Kinome screening using MIBs; Examining tumor tissues by IHC and immunoblot*
Funding Support:	*N/A*

Indiana University

Name:	*Wade Clapp, M.D.*
Project Role:	Subcontract PI
eBRAP ID:	*dclapp*
Nearest person month worked:	*1*
Contribution to Project:	*Regulatory activity involved in the genetic intercrosses, laboratory infrastructure, and strategic planning of murine models to validate the Group PAKs*
Funding Support:	*N/A*
Name:	*Su-Jung Park, Ph.D*
Project Role:	Associate Professor
eBRAP ID:	
Nearest person month worked:	*1*
Contribution to Project:	*Generation and Maintenance of murine models; Genotyping.*
Funding Support:	*N/A*
Name:	*Li Jiang, BME*
Project Role:	Research Technician
eBRAP ID:	
Nearest person month worked:	*3*
Contribution to Project:	*Maintains the genetically modified murine models; Conducts PCR; Assists in mice dissection and ABR testing.*
Funding Support:	*N/A*

Other Support

Chernoff, Jonathan

Remaining salary support from institutional sources.

ACTIVE

IRG-92-027-18 (PI: Chernoff)   1/1/2012 - 12/31/2015   NA
ACS   calendar
Institutional Research Grant
This is an Institutional Grant from the American Cancer Society that provides start-up funds for promising junior investigators to initiate innovative laboratory, translational, clinical and population research.
Procuring Contracting/Grants Officer: Virginia Krawiec, Extramural Grants Dept., 350 Williams St., Atlanta, GA 30303, 404-329-7612

R01 NS066927 (PI: Li, Vanderbilt Univ.)   3/1/2015 - 2/29/2020 NIH
                                                                                1.20   calendar
Pathophysiology of Conduction Block in HNPP
This project is a subcontract to Vanderbilt University.
Procuring Contracting/Grants Officer: Roddy Smith, Neurology Dept, 1161 21st Ave. South, Nashville, TN 37203, 615-936-8950

R01 CA142928 (PI: Chernoff)   1/1/2015 - 12/31/2019
NIH   2.40   calendar
Targeting the Kinome in Neurofibromatosis Type 1
The major goals of this project are to determine: 1) What is the status of the kinome in NF1-/- Schwann cells and mast cells, and how does this change upon Mek, Akt/mTOR, or Pak inhibition?; 2) What is the cellular basis for Pak's function in NF1-related tumors? and 3) Are small molecule Pak inhibitors effective in preclinical models of NF1? Can whole kinome analysis predict pathways for drug resistance in treated mice?
Procuring Contracting/Grants Officer: Emily Tran, OGA 9609 Medical Center Dr., West Tower, 2nd Flr., Rockville, MD 20850, 240-276-6324

W81XWH-14-1-0141 (PI: Chernoff)   5/15/2014 - 5/14/2017
Army   1.20   calendar
Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease
The major goals of this project are: 1) Does Pak signaling influence NF2-related pathology in vivo?; and 2) Are small molecule inhibitors of Pak effective in preclinical models of NF2?
Procuring Contracting/Grants Officer: Jodi Cardoza, US AMRAA, 820 Chandler St., Fort Detrick, MD 21702 (301) 619-2693

P30 CA006927 (PI: Fisher)   7/21/2011 - 6/30/2016
NIH   1.80   calendar
Comprehensive Cancer Center Program at Fox Chase
The major goal of this Cancer Center Support Grant is to provide partial salary support for professional personnel, including senior and program leadership, administration, planning and evaluation, and developmental funds, as well as support for 4 established peer-reviewed Research Programs, 12 Shared Research Resources and 1 Support Element.
Procuring Contracting/Grants Officer: Emily Tran, OGA 9609 Medical Center Dr., West Tower, 2nd Flr., Rockville, MD 20850, 240-276-6324

R01 CA148805 (PI: Chernoff / Testa)   3/1/2011 - 2/29/2016
NIH   0.96   calendar
The Role of p21-Activated Kinases in Malignant Mesothelioma (Multi-PI)
The major goals of this project are: 1) To determine the role of Pak in NF2-related signaling in MM cells; and 2) To determine the cellular and molecular basis for Pak signaling in MM-related pathology in vivo.
Procuring Contracting/Grants Officer: Emily Tran, OGA 9609 Medical Center Dr., West Tower, 2nd Flr., Rockville, MD 20850, 240-276-6324

OVERLAP
None

COMPLETED
K22 CA160725 (PI: Astsaturov) – NIH
C06 RR022050

Clapp, D. Wade

<u>**CURRENT**</u>
W81XWH-12-1-0155 (PI: Kropf)   05/15/13-05/15/16
Army   0.12 calendar
NFRP Neurofirbromatosis Clinical Trials Consortium
Goals: Implement the clinical proposal; collect and transmit the data at Riley Hospital for Children at Indiana University Health. Participate in results preparation of the NF Consortium Infrastructure for presentation and publication.
Specific Aims:
Primary Aim: To estimate the objective response rate to Cabozantinib at 12 months in adults with NF1 plexiform neurofibromas by volumetric MRI imaging.
Secondary Aims:

1. To further assess the tolerability and toxicity of Cabozantinib in patients with NF1
2. To estimate the objective response rate of up to 2 non-target plexiform neurofibromas to Cabozantinib by MRI
3. To determine the quality of life (QOL) response to Cabozantinib in patients with NF1 plexifrom neurofibromas
4. To access activity of Cabozantinib on mast cell activity by mast cell culture and FACS
5. To describe changes by flow cytometry in peripheral blood monocyte counts, circulating endothelial cells, and plasma angiogenic factors during treatment with Cabozantinib
6. To describe the baseline and change in 17 circulating cytokine factors related to proliferating cells
7. To characterize the pharmacokinetic profile of Cabozantibin in this population

Program Officer: Naba Bora, 1077 Patchel St. Fort Detrick, Frederick, MD, 21702-9218

R01 CA155294 (PI: Clapp)   02/01/11-12/13/15
NIH/NCI   0.91 calendar
Genetic Therapy for Fanconi Anemia
Goals: The goals of this application are to develop lentiviral mediated vectors applicable for treatment of FANCC and to develop novel methodologies to enhance the engraftment of murine and human hematopoietic stem/progenitor cells.
Specific Aims:
Aim 1. To finalize the development of a clinically applicable lentiviral vector and test functional expression of the transgene in immortalized lymphoblasts and human FANCC-/- myeloid progenitors.

> a) Generate recombinant lentiviral constructs that encode for expression of the FANCC cDNA driven by the human elongation factor (EF) 1α and the human phosphoglycerate kinase (PKG) promoters with and without the chicken HS4 insulator elements.
> b) Test functional expression of the transgenes in human FANCC deficient immortalized lymphoblasts.
> c) Evaluate functional expression of the FANCC transgene in human FANCC-/- myeloid progenitors.

Aim 2. To use a novel foamyviral envelope to optimize the transduction efficiency of wild-type and FANCC-/- human hematopoietic progenitor and SRC in NSG mice.

> a) Determine the transduction efficiency of LV pseudotyped with the PFV envelope on CD34+ cells.
> b) Evaluate transduction of hydroxyethyl starch (HES)-processed hematopoietic cells.
> c) Monitor gene transfer in primary FANCC-/- progenitors and SRC in NSG mice.
> d) Test the role of human mesenchymal stem cells (MSCs) in enhancing engraftment of corrected human FANCC-/- HSCs in NSG mice.

Aim 3. To optimize non-genotoxic strategies of HSC engraftment utilizing a novel FA murine model that develops spontaneous bone marrow failure (BMF).

  a) Determine whether myelopreparation is required in hypoplastic FA knockout mice.

  b) Utilize IFN-γ as an agent to enhance exogenous engraftment of donor HSCs.

  c) Evaluate to what extent normal MSCs enhance engraftment of genetically corrected donor Fancc-/- BM cells.

Program Official: Mary Katherine DeFilippes, 9609 Medical Center Dr., BG 9609 RM 4W224, Rockville,MD, 20850

P50 NS052606   08/15/10-07/31/15

NIH/NINDS/UTSW   0.29 calendar

Role of hematopoietic microenvironment in plexiform neurofibroma progression

Goals: To Evaluate the role of monocyte/macrophages in plexiform neurofibroma formation

Specific Aims:

Aim 1. Evaluate the role of α4β1 integrin signals in promoting murine mast cell adhesion to endothelium, mast cell proliferation, and secretory factors that promote neoangiogenesis and tumor progression.

  a) Examine the role of α4 in modulating human and murine Nf1+/- mast cell/endothelial cell interactions in vitro.

  b) Determine whether disruption of α4 in Nf1+/- hematopoietic cells is sufficient to prevent or delay tumor progression in the plexiform neurofibroma murine model.

Aim 2. To test the hypothesis that MMP-9 cooperates with c-kit in neurofibroma progression.

  a) Intercross Krox20; Nf1flox/- with MMP9-/- mice.

  b) Evaluate peripheral blood mast cell and other myeloid lineages as a biomarker.

  c) Determine circulating c-kit ligand, M-CSF1, TGF-β, MCP-1, VEGF using a multiplex array.

  d) Determine tumor progression using FDG-PET and harvest of cohorts at 6-12 months.

Aim 3. To test the hypothesis that macrophages are key effectors required for neurofibroma progression.

  a) Examine human NF1 and unaffected adult control macrophage migration, de novo synthesis of cytokines and neoangiogenic promoting activity in HUVECS.

  b) Transplant Krox20; Nf1flox/- mice with: Nf1+/-, EGFP, Nf1+/-;CSF1-/-, WT, or CSF1-/- bone marrow cells.

Program officer: Jill A. Morris, 6001 Executive Blvd MSC, Room 2133, Bethesda, MD, 20892

(PI: Clapp)

05/01/14-04/30/17 Children's Tumor Foundation   1.20 calendar

Experimental Therapeutics targeting the NF2 kinome: an integrated rational approach

Goals: The goal of this application is to bring a multidisciplinary group of investigators together in a vertically integrated program that allows consistency of screening approaches, utilizes both established and newly developed cellular and murine models of NF2-associated tumors, allows validation of the mechanisms of action of drugs and investigation of the biologic result of target inhibition in both VS and meningiomas as well as exploration of the cellular compensatory events influencing drug response with the ultimate goal of developing clinical studies that have the highest chance of success for patients.

Specific Aims:

Aim 1: Utilize NF2/(Nf2)-deficient schwannoma and meningioma cells to screen:

  a) Established FDA-approved drugs and targeted therapeutics in the clinical pipeline that have rational linkage to Nf2-dependent pathways in collaboration with industry partners

  b) A library of late-stage and FDA-approved drugs in collaboration with the National Center for Advancement of Translational Science (NCATS) to allow an unbiased drug screen

Aim 2: Utilize genetically engineered (VS) and xenograft (meningioma) models to test drugs that meet in vitro go/no go criteria:

  a) Vestibular schwannomas: PeriostinCre; Nf2flox/flox mice will be treated with drugs successful in the in vitro screen against schwannomas. The preclinical endpoints will be

restoration of baseline hearing in 50% of mice and 50% mean reduction in paraspinal tumor volume by MRI and at autopsy in 75% of mice compared to mice treated with a vehicle control.

b) Meningiomas: Ben-Men-1 cells (benign NF2-deficient meningioma cells) will be implanted intracranially and mice will be treated with drugs successful in the in vitro screen against meningiomas. The preclinical endpoint will be 50% mean reduction in tumor volume by MRI and luciferase activity in 75% of mice compared to mice treated with a vehicle control.

Aim 3: Identify at least 1 drug to move forward for a clinical trial.

a) Drs. Blakeley and Welling will be involved in all stages of go/no-go decision making and will design the appropriate clinical trial for the agent(s) with the best portfolio based on:

a) Acceptable safety profile

b) Efficacy in vitro and in vivo against cell culture models of schwannoma and meningioma - Preference will be for joint activity against VS and meningioma and for drugs with delineated mechanisms in NF2 tumors

c) Drugs that are in active clinical development with an industry partner

Aim 4: Delineate cell and molecular validation of the best performing experimental compounds

a) Identify cellular mechanisms of action of drugs in tumors in vitro and in vivo

b) Utilize immunohistochemistry and western blot to validate inhibition of the biochemical target in tumorigenic cells of the in vivo models

c) Conduct kinome analyses on drugs that pass the established go/no go criteria for at least one in vivo model to evaluate patterns of adaptation to therapy and the differences between meningioma and VS therapeutic responses

d) Confirm synthetic lethality via genetic confirmation with shRNA

Program officer: Annette Bakker, 95 Pine Street, 16th Floor, New York, NY, 10005

W81XWH-14-1-0141 (PI: Chernoff)   5/15/2014 -
5/14/2017 Army   1.20 calendar
Testing the Role of p21-Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease
Goals: The objective of this project is to evaluate Pak as a therapeutic target in NF2.
Specific Aims:
Aim 1: Does Pak signaling influence NF2-related pathology *in vivo*? If group A Paks are important for growth and motility signaling in cells lacking NF2, then loss of Pak function should slow or prevent pathologies associated with loss of the *Nf2* gene in mice. We have already constructed $Pak1^{-/-}$ and $Pak2^{flox/flox}$ mice, as well a targeted transgenic knock-in mouse that conditionally expresses the PID, a peptide inhibitor of all group A Paks, in any tissue expressing Cre recombinase. We will cross these transgenic mice, as well as our existing *Pak1* knock-out mice, with Periostin (*Postn*)-*Cre*; $Nf2^{flox/flox}$ mice to answer the following questions:

a) Does loss of the *Pak1* or *Pak2* gene alone ameliorate loss of the *Nf2* gene in schwannoma formation? What signaling pathways mediate these effects?

b) Does suppression of group A Pak kinase function by a genetically encoded peptide inhibitor ameliorate loss of the *Nf2* gene *in vivo*? What signaling pathways mediate these effects?

c) What is the basal state of the kinome in NF2 schwannomas and how is the kinome in Schwann cells or tumors reprogrammed upon loss of Pak function?

Aim 2: Are small molecule inhibitors of Pak effective in preclinical models of NF2? Selective small molecule Pak inhibitors have recently entered clinical trials. In NF2 cell culture and in xenograft settings, such small molecule inhibitors have proven effective in preventing tumor growth and in inhibiting Merlin signaling. We will test three of the most advanced Pak inhibitors to determine if they are beneficial in improving auditory function in *Postn-Cre;* $Nf2^{flox/flox}$ mice and in reducing tumor growth:

a) Do inhibitors of group A Paks restore normal hearing and/or promote tumor regression in *Postn-Cre;* $Nf2^{flox/flox}$ mice?

b) Do pan-Pak (group A plus group B) inhibitors restore normal hearing and/or promote

tumor regression in *Postn-Cre*; *Nf2$^{flox/flox}$* mice?
Program officer: Scott Linton, U.S. Army Medical Research and Material Command, Fort Detrick, Maryland, 21702-5012

(PI: Clapp)
07/15/14-07/14/15 Children's Tumor Foundation   0.60 calendar
Children's Tumor Foundation /Neurofibromatosis Therapeutics Acceleration Program (NTAP)
Drug Discovery Initiative Neurofibromatosis Preclinical Consortium Center
Goals: The goal is to identify improved ways to monitor response of plexiform neurofibromas to therapy.
Specific Aims:
Aim 1: The first study will be to test MEK inhibitor (AZD 5362) as a single agent and then in combination with the AKT inhibitor (AZD 6244) in order to determine the effects these agents have on the plexiform neurofibromas.
Aim 2: In continuation with our previous funded studies with the AKT inhibitor know as, AZD 6244, and in collaboration with Dr. Brigette Widemann, we will examine alternate dosing schedules in order to determine the proper dosing for long term treatments.
Aim 3: These studies will utilize the transforming growth factor-beta inhibitor, LY2157299, in order to identify TGF-β as novel molecular target to inhibit neurofibroma formation. Due to our success translating preclinical results to clinical trials, we foresee this as a potential drug that could be accelerated to in human testing if successful in our preclinical work
Aim 4: We hypothesize that treatment with PI3K/dual mTORC1/2 inhibitor, LY3023414, will provide significant tumor reduction of plexiform neurofibromas *in vivo* and reduce contributions to tumor progression from NF1$^{+/-}$ mast cells.
Program Officer: Annette Bakker, 95 Pine Street, 16th Floor, New York, NY, 10005

5/01/14-04/31/16

Koltan Pharmaceuticals   0.60 calendar
Testing the pharmokenetic effect anti-C-KIT antibody has on the Genesis of Plexiform Neurofibromas
Goals: To Test the effect anti- c-kit antibody has on mast cells and the genesis of plexiform neurofibromas
Specific Aims:
Aim 1: The first study will be to test the effects of KIT inhibition has on mast cell recruitment to skin in response to Stem Cell Factor (SCF).
Aim 2: The second study will be to compare the effects of KIT inhibition on NF1-deficient and wild-type mast cell function utilizing *in vitro* techniques.
Program Officer: Rich Gedrich, 300 George Street, Suite 350, New Haven, CT, 06511

07/01/14-08/31/15

Koltan Pharmaceuticals   0.12 calendar
Testing the Pharmokenetic effect KTN3379 has on the Genesis of Plexiform Neurofibromas
Goals: To Test the effect KTN3379 antibody has on Schwann Cells and the genesis of plexiform neurofibromas
Specific Aims:
Aim 1: The first study will be to test the cellular function of ErBb3 inhibition on primary murine Nf1-/- Schwann cells utilizing KTN3379 and 2C2.
Aim 2: In the subsequent study of this research proposal, our group will perform *in vivo* studies utilizing our Nf1 murine model in order to test the effects of ErbB3 inhibition on the proliferation of Schwann cells.
Program Officer: Rich Gedrich, 300 George Street, Suite 350, New Haven, CT, 06511

Developmental HyperActive Ras Tumor SPORE

Goals: The overall goal of this SPORE is to implement effective targeted molecular therapies for neoplasms and cancers characterized by *NF1* mutations by conducting integrated, mechanistically based translational research. This highly-qualified, collaborative group will address the following overarching objectives:

1. To evaluate novel therapeutics in validated preclinical models and in the treatment of patients with NF1.
2. To Identify risk factors of individuals with NF1 to acquire spontaneous and treatment-associated second malignancies
3. To decrease tumor associated morbidity and mortality of patients with NF1

Program Officer: Igor Kuzmin, 9609 Medical Center Drive, Room 3W112, MSC 9726, Bethesda, MD, 20892

OVERLAP

None

COMPLETED

W81XWH-12-1-0120, NF110107, (PI: Clapp)
R01 CA074177
R01 CA138237

Other Support

Chow, Hoi Yee

Salary support from institutional sources.

ACTIVE
W81XWH-14-1-0141 (PI: Chernoff)   5/15/2014 -
5/14/2017 Army   6.00   calendar
Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered
Murine Model that Closely Phenocopies Human NF2 Disease
The major goals of this project are: 1) Does Pak signaling influence NF2-related pathology in vivo?; and 2) Are
small molecule inhibitors of Pak effective in preclinical models of NF2?
Procuring Contracting/Grants Officer: Jodi Cardoza, US AMRAA, 820 Chandler St., Fort Detrick, MD 21702
(301) 619-2693

OVERLAP
None

OTHER SUPPORT

Park, Su-Jung

ACTIVE
W81XWH-14-1-0141 (PI: Chernoff)   5/15/2014 -
5/14/2017 Army   1.20   calendar
Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered
Murine Model that Closely Phenocopies Human NF2 Disease
The major goals of this project are: 1) Does Pak signaling influence NF2-related pathology in vivo?; and 2) Are
small molecule inhibitors of Pak effective in preclinical models of NF2?
Procuring Contracting/Grants Officer: Jodi Cardoza, US AMRAA, 820 Chandler St., Fort Detrick, MD 21702
(301) 619-2693

OVERLAP
None

COMPLETED
W81XWH-12-1-0120, NF110107, (PI: Clapp)